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In Silico Study of the potency of *Drynaria rigidula* (Sw.) Bedd. (Polypodiaceae) compounds in breast cancer therapy targeting AKT1 protein pathways

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ABSTRAK

Kanker payudara adalah kanker kedua yang paling umum di dunia dan menempati peringkat pertama di Indonesia. Salah satu jalur utama yang terlibat dalam kanker payudara adalah mekanisme PI3K/Akt. Senyawa alami dengan potensi yang belum banyak diteliti adalah Drynaria rigidula *(Sw.) Bedd., satu jenis paku Polypodiaceae yang berasal dari Indonesia dan biasa digunakan dalam pengobatan tradisional sebagai obat herbal. Oleh karena itu, penelitian ini bertujuan untuk mengeksplorasi potensi Drynaria rigidula sebagai agen anti-kanker payudara. Metode yang digunakan dalam penelitian ini adalah analisis in silico dengan mengumpulkan data dari beberapa server web seperti SwissADME (https://www.swissadme.ch/) dengan parameter aturan lima Lipinski, Veber, Egan, dan Way2Drug untuk aktivitas biologis. Protein yang digunakan dalam penelitian ini adalah AKT1 (PDB ID: 6HHF), yang diperoleh dari database RCSB. Analisis* docking *molekuler dilakukan menggunakan perangkat lunak PyRx dengan visualisasi dilakukan di Biovia Discovery Studio. Hasilnya menunjukkan bahwa beberapa senyawa, seperti asam 3,4-dihidroksibenzoat, fern-9(11) ene, Stigmasterol, dan Campesterol, memiliki nilai RMSD < 3,0 Å dan afinitas pengikatan masing-masing -9,4, -9,2, -7,6, dan -7,6. Hasil ini dibandingkan dengan ligan kontrol AZD5363 dan doksorubisin, yang memiliki afinitas pengikatan masing-masing -8,3 dan -7,4. Oleh karena itu, hasil docking menunjukkan bahwa senyawa dari Drynaria rigidula diprediksi memiliki potensi sebagai agen anti-kanker.*

Kata kunci: AKTI, Anti-kanker,* Drynaria rigidula*, Pteridofita

ABSTRACT

Breast cancer is the second most prevalent cancer globally. One of the key pathways involved in breast cancer is the PI3K/Akt mechanism. A natural compound with potential that has not been extensively studied is Drynaria rigidula (Sw.) Bedd., a Polypodiaceous fern native to Indonesia and is commonly used in traditional medicine. This study aims to explore the potential of Drynaria rigidula as an anti-breast cancer agent. The method used in silico analysis by collecting data from several web servers such as SwissADME (https://www.swissadme.ch/) with the parameters Lipinski's rule of five, Veber, Egan, and Way2Drug for biological activity. The protein used in this study is AKT1 (PDB ID: 6HHF), obtained from the RCSB database. Molecular docking analysis was conducted using PyRx software with visualization performed in Biovia Discovery Studio. The results showed that several compounds, such as 3,4-dihydroxybenzoic acid, fern-9(11) ene,

Stigmasterol, dan Campesterol, had RMSD values < 3.0 Å and binding affinities of -9.4, -9.2, -7.6, and -7.6 respectively. These results were compared with the control ligand AZD5363 and doxorubicin, which had a binding affinity from each other are -8.3 and -7.4. Therefore, the docking results indicate that compounds from Drynaria rigidula are predicted to have potential as anti-cancer agents.

Keywords: **AKTI, Anti-cancer, Drynaria rigidula, *Pteridophytes***

INTRODUCTION

Cancer is the second leading cause of death worldwide, following cardiovascular diseases (Cancer-WHO, 2022). Additionally, according to WHO data (2022), breast cancer is the second most prevalent cancer globally and the most prevalent in Indonesia. Several factors contribute to the high incidence of cancer, particularly breast cancer, in Indonesia, including unhealthy lifestyles, limited access to cancer treatment, low public awareness, slow early detection and prevention, and the inability of conventional cancer drugs like chemotherapy to combat cancer cell resistance (Sung et al., 2021).

Conventional treatments, such as chemotherapy, have side effects that can be harmful if administered over a long period. These include damage to living cells, death of non-cancer cells, nausea, dizziness, and hair loss (Wang et al., 2012). There are many cancer treatments available besides chemotherapy, such as Monoclonal Antibody drugs that can target cancer cells. However, the use of these drugs is limited due to their high cost (Globocan, 2022).

Indonesia's rich biodiversity is considered as a potential source for further development and research of natural bioproducts. Several plants are believed to have medicinal properties to treat various diseases. One among which is *Drynaria rigidula*, a Polypodiaceous epiphytic fern commonly found in Indonesia. According to Wirdayanti & Nery Sofiyanti (2019), species of ferns from the family Polypodiaceae have been studied for their use as traditional medicine sources in various countries. The leaves of ferns from the genus *D. rigidula* contain bioactive compounds such as flavonoids, triterpenoids, saponins, phytoecdysteroids, and others (Ari S. Nugraha et al., 2013). *D. rigidula* ferns are found throughout Indochina, western Malesia (including Java), Wallacea, Papua and Northern Australia. In Indonesia it has been used traditionally by local people as a medicinal plant (Nugraha et al., 2019).

Several compounds found in *D. rigidula*, such as ferulic acid, chlorogenic acid, and caffeic acid, have high antioxidant properties. Additionally, Dicholoromethane extracts from *D. rigidula* have shown cytotoxic activity against breast cancer cell (MCF-7) due the presence of kaemferitin (Nugraha et al., 2019).

One characteristic of breast cancer cells is the high expression of the AKT1 protein (Protein Kinase B 1), which plays a role in cell growth and proliferation. Additionally, the AKT1 protein is involved in the inactivation of proapoptotic proteins such as Bad (Bcl2-antagonist cell death) and other proteins like p53, which are involved in cell death (Gao et al., 2021; Zhang et al., 2013). The structure of AKT1 consists of main domains, namely the pleckstrin homology (PH) domain at the N-terminal end, the serine/threonine-specific kinase domain, and the regulatory domain at the C-terminal end. AKT1 activation

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occurs through phosphorylation mediated by phosphatidylinositol 3-kinase (PI3K). Dysregulation of AKT1 function has been associated with various pathological conditions, including cancer, where mutations in the AKT1 gene can cause abnormal enzyme activation, contributing to tumor development and uncontrolled cell growth. AKT1 is a potential target in the development of breast cancer therapies, and AKT1 inhibitors need to be studied to suppress tumor growth and increase sensitivity to existing therapies.

Furthermore, AKT1 is a protein involved in the PI3K/AKT/mTOR pathway. This pathway is important in regulating cell growth, proliferation, metabolism, and survival. It also plays a crucial role in the activation and inactivation of the p53 protein, which is involved in breast cancer cell apoptosis. Therefore, this study aims to analyze and predict anti-cancer compounds found in *D. rigidula*. in breast cancer cells, specifically targeting the AKT1 protein.

METHODOLOGY

Observation and identification

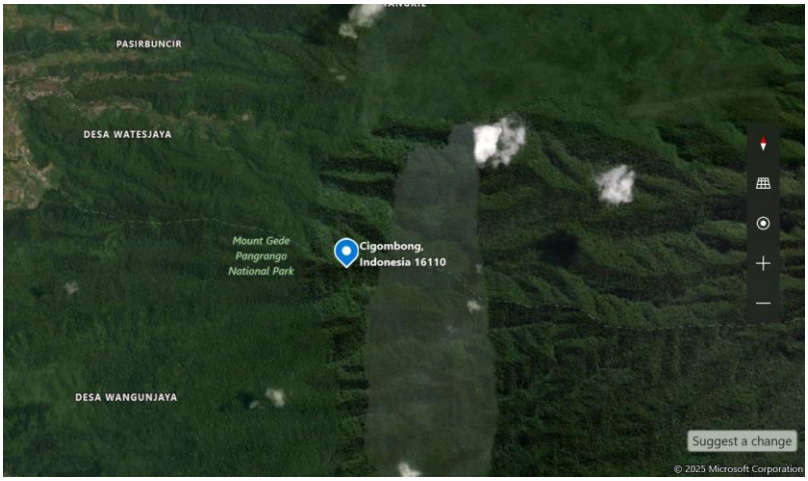

FIGURE 1. Map of observation track of ferns in PPKA Bodogol, West Jawa.

The observation of ferns was conducted at PPKA Bodogol, West Jawa, by examining several plant species. The identification process was carried out by matching field photographs (**Figure 2**) with the Herbarium of the Biology Department at Universitas Negeri Jakarta (UNJ) and verifying them with the herbarium curator. The nomenclature follows POWO (2025).

Compound screening

Natural compounds from *D. rigidula* were obtained from Nugraha et al., 2013 & Nugraha et al., 2019. The results of the natural compound search were then analyzed using SwissADME (https://www.swissadme.ch/) with the parameters Lipinski's rule of five, Veber, and Egan. The higher drug-likeness ratings will indicate that the molecules have better potential as drug candidates (Rai et al., 2023). Additionally, toxicity levels were measured using ProTox 3.0 (https://tox.charite.de/protox3/#), to determine whether the compounds could be harmful to the body (Lipinski, 2004; Way2Drug - Main, n.d.; Wei et al., 2022; Yang et al., 2019; Nugraha et al., 2013; Nugraha et al., 2019.).

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Prediction of biological activity

The prediction of ligand biological activity was conducted using the PASS server (https://www.way2drug.com/passonline/). The PASS server can identify several potential compounds by evaluating the Probable Activity (Pa) value > 0.7. Based on this value, the biological activity of a compound can be predicted (Way2Drug - Main, n.d.).

Protein screening

The selection of proteins was based on their biological activity in breast cancer cells. The Akt1 protein was chosen from the PDB (PDB ID: 6HHF) as the receptor present in breast cancer cells. The protein was then prepared using Biovia Discovery Studio 2021 to remove native ligands and water molecules. Subsequently, the protein was converted into a pdbqt file for the docking process (BIOVIA Discovery Studio | Dassault Systèmes, n.d.; RCSB PDB: Homepage, n.d.).

Molecular docking

Molecular docking was performed using Autodock Vina on the AKT1 protein (PDB ID: 6HHF) from the RSCB database, with ATP (CID: 5957) as the native ligand and AZD5363 (CID: 2522436) as the positive control from PubChem. Ligand preparation was conducted using PyRx software before docking with the AKT1 protein. Doxorubicin was used as the positive control for conventional chemotherapy drugs. The grid center used was X: 15.490, Y: 2.559, Z: 11.064, and Dimension (Angstrom): X: 38.176, Y: 33.382, Z: 44.080. The docking results were visualized using Biovia Discovery Studio (Dallakyan & Olson, 2015).

RESULTS AND DISCUSSION

Observation and identification

The observation results led to the selection of *Drynaria rigidula*, which was identified at the Herbarium of the Biology Department at Universitas Negeri Jakarta (UNJ). This plant was found at an altitude of 800 meters above sea level (masl). It will then be analyzed *in silico* using various literature studies and web servers to assess its potential as an anticancer agent.

DOI: 10.21009/bioma.v20i2.49340

FIGURE 2. *Drynaria rigidula* individual sampled at PPKA Bodogol, West Jawa, perching as high as 15 m above tree canopy as nest Drynarioid fern.

Compound screening

The screening results identified seven compounds based on ADME analysis: 3,4-dihydroxybenzoic acid, hop-22(29)-ene, fern-9(11) ene, kaempferol-3,7-di-O-α-l-rhamnopyranoside, stigmasterol, campesterol, and β-sitosterol. The screening results will guide further computational and experimental studies, focusing on the most promising compounds for docking and in vitro validation. The results from the ADME screening analysis indicate that several compounds have potential as anti-cancer agents. However, compounds with high toxicity levels require special attention.

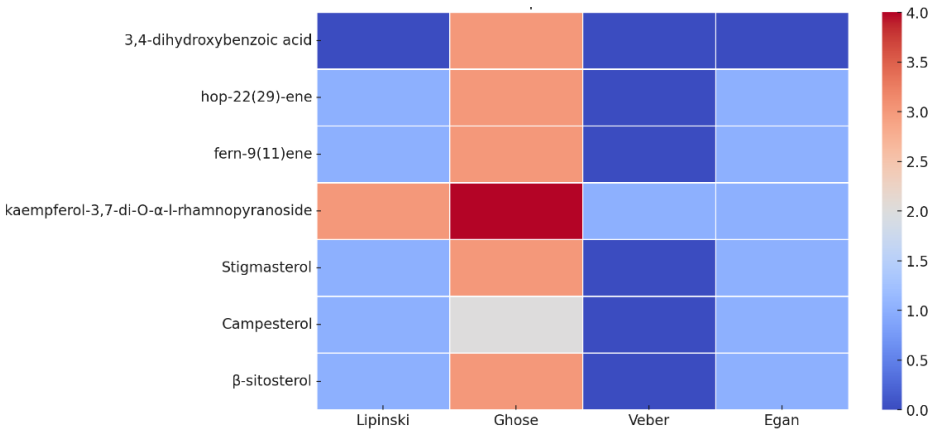

FIGURE 3. Screening results of *Drynaria rigidula* compounds using the Lipinski, Ghose, Veber, and Egan parameters (The level 0 – 5 indicate the level of violation in every compound).

The results in **Figure 3** indicate that 3,4-dihydroxybenzoic acid is the most promising compound based on ADME screening. This compound exhibits zero violations in Lipinski, Veber, and Egan rules, with only a high Ghose violation, making it a good candidate for further analysis due to its potential drug-like properties and favorable pharmacokinetics. Conversely, hop-22(29)-ene, fern-9(11) ene, stigmasterol, campesterol, and β-sitosterol are less ideal, as they violate one Lipinski rule, have Ghose violations of two or more, and fail Egan's criteria. These factors may limit their oral

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bioavailability and absorption. Among all, kaempferol-3,7-di-O-α-l-rhamno- pyranoside is the least ideal compound, exhibiting multiple violations across the Lipinski (3), Ghose (4), Veber (1), and Egan (1) rules, suggesting poor pharmacokinetic properties. These findings help prioritize compounds for further docking and in vitro validation, focusing on those with minimal ADME violations.

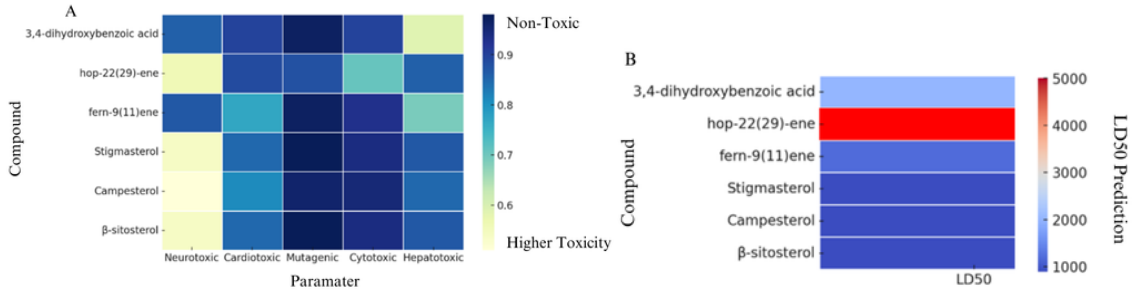

FIGURE 3. A. Screening results of *Drynaria rigidula* compounds for the probability of inducing toxicity (Closer to 1: Indicates inactive toxicity, meaning the compound is less toxic in that category. Closer to -1: Indicates active toxicity, meaning the compound exhibits significant toxic effects in that category. Values around 0 suggest moderate or uncertain toxicity) and B. The LD50 prediction analysis.

The results of **Figure 3A** shows that the heatmap illustrates the toxicity profiles of various compounds across different toxicity types, including neurotoxicity, cardiotoxicity, mutagenicity, cytotoxicity, and hepatotoxicity. The 3,4-Dihydroxybenzoic acid shows high values (~0.86–0.97) in most categories, indicating low toxicity, but it has a slightly lower score in hepatotoxicity (0.59), suggesting a relatively higher risk for liver toxicity. Hop-22(29)-ene has a low neurotoxicity score (0.56), indicating potential toxicity in this aspect, but remains largely inactive in other categories. Fern-9(11)-ene shows a lower score in hepatotoxicity (0.69), suggesting moderate liver toxicity risk. Stigmasterol, Campesterol, and β-Sitosterol have high mutagenicity and cytotoxicity values (~0.94–0.98), suggesting they are inactive in these aspects and unlikely to cause genetic or cellular damage.

The results of **Figure 3B** shows the LD50 values of various compounds. LD50 (Lethal Dose 50%) refers to the dose of a substance that causes death in 50% of a test population (typically laboratory animals) and is expressed in mg/kg of body weight. The color gradient indicates toxicity levels based on LD50 values: Red (hop-22(29)-ene) represents the highest LD50 (5000 mg/kg), meaning it is the least toxic compound since a higher dose is required to reach lethal effects. Dark blue represents lower LD50 values (~890 mg/kg), indicating higher toxicity as smaller doses can cause lethality. Light blue (e.g., 3,4-dihydroxybenzoic acid) indicates moderate LD50 values (~2000 mg/kg).

Biological activity

The prediction of probable activity (PA) and probable inactivity (PI) values was determined using the PASS server. The data in **Table 2** show that almost all compounds found in *D. rigidula*. have the potential to act as apoptosis agonists. This is significant for cancer treatment. Apoptosis agonists (or apoptosis inducers) were chosen by the researchers because they align with the target protein, AKT1, which plays a role in the apoptosis mechanism in cancer cells.

DOI: 10.21009/bioma.v20i2.49340

TABLE 1. Biological activity of active compounds derived from *D. rigidula*

Numb.	Compound Name	Biological Activity Possible Activity Score (PA Score)			
		Antineoplastics	**TP53 Expression**	**JAK2 Expression Inhibitor**	**Apoptosis agonist**
1.	3,4-dihydroxybenzoic acid	0.41	0.70	0.82	0.46
2.	hop-22(29)-ene	0.93	0.45	0.37	0.85
3.	fern-9(11) ene	0.83	0.34	0.34	0.86
4.	Stigmasterol	0.53	0.59	0.49	0.75
5.	Campesterol	0.40	0.58	0.54	0.67
6.	β-sitosterol	0.41	0.55	0.46	0.20

Table 2 presents biological activity data for six different compounds, analyzing their potential activities across four key cancer-related mechanisms. Each compound is evaluated based on its Possible Activity Number (PA) in relation to antineoplastic properties, TP53 expression, JAK2 expression inhibition, and agonist apoptosis activity. The compounds include 3,4-dihydroxybenzoic acid, hop-22(29)-ene, fern-9(11)-ene, stigmasterol, Campesterol, and β-sitosterol, with their PA values ranging from 0.20 to 0.93 across different activities.

Looking at the data more closely, hop-22(29)-ene shows the highest antineoplastic activity (0.93), while fern-9(11)-ene and hop-22(29)-ene demonstrate strong-apoptosis agonist properties (0.86 and 0.85 respectively). 3,4-dihydroxybenzoic acid exhibits the highest JAK2 expression inhibitor activity (0.82) and TP53 expression (0.70) among all compounds. In contrast, β-sitosterol shows relatively moderate activity across most parameters but notably low apoptosis agonist activity (0.20), while Campesterol and stigmasterol demonstrate moderate activity across all parameters without any particularly outstanding values. Similar studies have shown that compounds like Stigmasterol and beta sitosterol can induce apoptosis, cell cycle arrest in various cancer cells, such as MCF-7, MDA-MB231, and HEPG2 (Liver Cancer) (Wang et al., 2023; Zhang et al., 2022).

A. Molecular Docking

After screening the best bioactive compounds for docking, docking was performed by evaluating the lowest binding affinity of each compound with the AKT1 protein complex. **Table 3** presents the results of molecular docking analysis, showing compounds with binding affinities from lowest to highest. These compounds interact with various key residues on the AKT1 protein, potentially serving as therapeutic agents to inhibit the protein's activity. β-sitosterol is not passed for the docking screening because it has a biological activity score below 0.7 for all parameters.

The RMSD value is used to determine whether a compound remains stable during docking analysis. Additionally, the RMSD value indicates conformational structural changes. If the RMSD value is < 2 Å, the compound is considered very good; however, if it exceeds 2 Å, the compound has undergone structural changes. These changes may prevent the compound from binding on the receptor as initially targeted by the researchers.

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TABLE 2. Docking Analysis Results on AKT1 Protein Receptor

Numb.	Compound	Binding Site	Rmsd		Binding Affinity
			Ub	Lb	
1	3,4-dihydroxybenzoic acid	Thr 211, Phe 209, Ser 205, G' 4k501	0.63	1.19	-9.4
2	fern-9(11) ene	leu 362, leu 384, lys 386, glu 365, ile 366, ser 381, phe 368, lys 385, Asp 387	0.82	1.30	-9.2
3	hop-22(29)-ene	Ile 366, Glu 365, Lys 377, Phe 368, Lys 385, Lys 386, Ser 381, Asp 387, Gln 390	1.88	9.14	-7.4
4	Campesterol	Tyr 18, Asn 54, Tyr 326, Val 271, Val 330, Asp 331, Leu 261, Asn 279, Ile 257, Thr 291, Ala 260, Leu 280, Lys 289, Ile 288, Phe 209, Leu 264, Leu 275	1.22	1.78	-7.6
5	Stigmasterol	Phe 209, Ala 260, Ile 257, Leu 264, Leu 275, Val 330, Tyr 18	0.42	1.01	-6.6
6	Native Ligand Control (AZD5363)	Tyr 315, Ala 317, Val 271, Val 320, Val 330, Tyr 18, Tyr 326, Lys 276, Unl 1	2.42	4.06	-8.3
7	Doxorubicin (Positive Control)	Gly 334, Ala 317, Val 330, Trp 333, Leu 316, Tyr 315, Tyr 418, Thr 312, Tyr 275, Leu 275, Val 320, Gly 294, Gly 327, Gly 501, Asn 54	1.34	1.98	-7.4

Compounds with the highest molecular docking analysis results, such as 3,4-dihydroxybenzoic acid with a binding affinity of -9.4, followed by other compounds like fern-9(11) ene and hop-22(29)-ene. However, hop-22(29)-ene compounds have very high RMSD values for lower bonds, indicating potential conformational changes. Therefore, compounds with both good upper and lower RMSD values and the best binding affinity and RMSD values are 3,4-dihydroxybenzoic acid (-9.4), Catechin fern-9(11) ene (-9.2), Campesterol (-7.6), and Stigmasterol (-6.6). The control for these compounds, using AZD5363 as native ligand and Doxorubicin as positive control, both achieved a good binding affinity score of -8.3 and -7.4 with an RMSD value below 2 Å.

Compounds such as Campesterol exhibit good hydrogen and hydrophobic bonding. They interact with several amino acid residues similar to those of the control ligand (Doxorubicin), such as VAL 330, Asn 54, Leu 275, Tyr 18. Meanwhile, other compounds like Stigmasterol show similarity in amino acid residues at Leu 275 with doxorubicin control. However, 3,4-dihydroxybenzoic acid, fern-9(11) ene, hop-22(29)-ene does not share amino acid residues with the native ligand but show similarity with other ligands.

In contrast, **Figure 4** shows the docking results between Akt1 and the positive control ligand, doxorubicin, which indicates a good binding affinity of -8. However, there is some unfavorable bump error in Leu 275. This result suggests that positive control may not always maintain stability with the target protein in cancer cells. However, based on the binding interactions and amino acid residues, doxorubicin shows good binding at the ATP binding pocket of Akt1. This evaluation highlights that doxorubicin may not perform as well as some compounds derived from *D. rigidula*.

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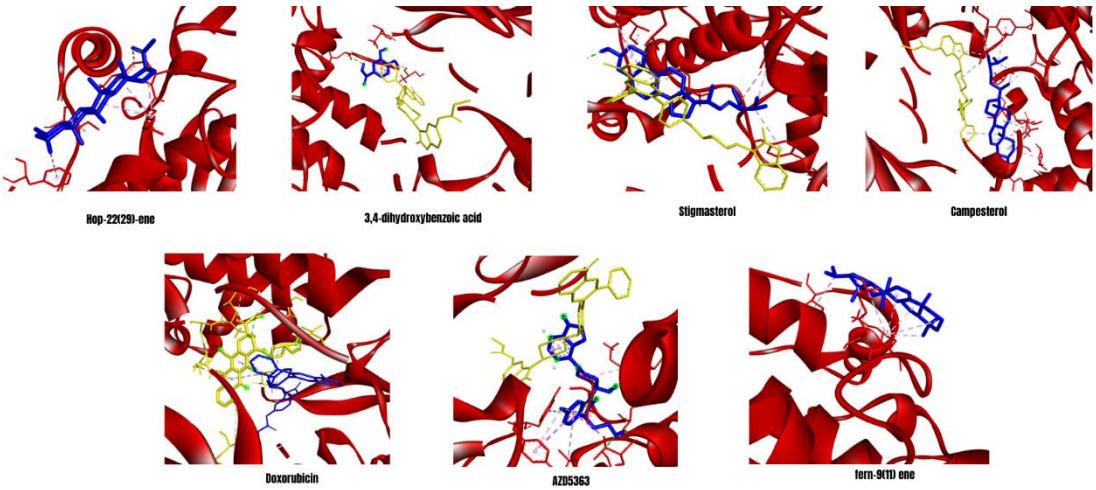

FIGURE 4. Docking results of *D. rigidula* compounds with AKT1 protein

The docking results for the AKT1 protein are ideally located at the ATP binding site in the center of the AKT1 protein (Widyananda et al., 2022; Zhang et al., 2013). The AKT1 protein binds to ATP at the kinase domain, which plays a role in substrate phosphorylation and subsequently transfers phosphate from the substrate to the cell. Therefore, one potential theory to inhibit AKT1 from proliferating cells is to block the ATP binding site on the protein. Consequently, in **Figure 4**, compounds such as Campesterol and Stigmasterol are unable to inhibit the ATP domain on AKT1 (Al-Bahlani et al., 2021). This is also evidenced by the RMSD results, which exceed 2 Å.

Several studies have also shown that one of the highly expressed proteins in T47D cells is the AKT1 protein (Widyananda et al., 2022). These studies explain that inhibiting the ATP domain with the AKT1 protein plays a role in inhibiting the proliferation of liver cancer cells (HEPG-2). This is also influenced by the fact that AKT1 is one of the key proteins involved in the PI3K/Akt pathway, so inhibiting AKT1 also hinders cancer cell proliferation. Other proteins that also play roles in the PI3K/Akt pathway, such as AKT2 and AKT3, are also important. However, these two proteins are more involved in inducing cell migration and invasion.

Meanwhile, in **Figure 5**, the 2D confirmation results also show that several compounds have bonds with the same amino acid residues. However, not all compounds are located at the expected site, which is the ATP binding pocket of AKT1. For instance, compounds such as fern-9(11) ene and hop-22(29)-ene do not bind well to the ATP binding pocket of AKT1.

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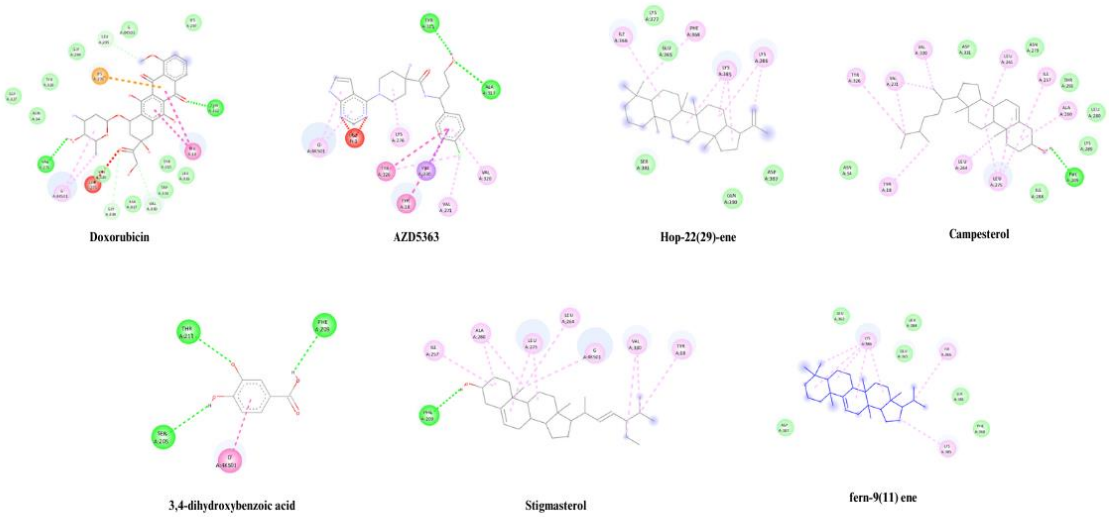

FIGURE 5. Interaction between protein-ligand and *D. rigidula* compounds on AKT1 protein

The docking results of AKT1 protein are ideal when located at the ATP binding site in the center of the AKT1 protein (Widyananda et al., 2022; Zhang et al., 2013). AKT1 protein binds to ATP at the kinase domain, which plays a role in substrate phosphorylation and subsequently transfers phosphate from the substrate to the cell. Therefore, one theory to inhibit AKT1 from proliferating cells is to block the ATP protein. In **Figure 4**, compounds such as Stigmasterol, Campesterol, and 3,4-dihydroxybenzoic acid are unable to inhibit the ATP domain on AKT1 (Al-Bahlani et al., 2021). This is also evidenced by RMSD results exceeding 2Å.

Several studies have shown that one of the highly expressed proteins in T47D cells is AKT1 (Widyananda et al., 2022). This research explains that inhibition of the ATP domain with AKT1 protein plays a role in inhibiting the proliferation of liver cancer cells (HEPG-2). This is also influenced by the fact that AKT1 is one of the important proteins in the PI3K/Akt pathway, so inhibiting AKT1 also inhibits cancer cell proliferation. Other proteins that also play a role in the PI3K/Akt pathway, such as AKT2 and AKT3, are also important. However, these two proteins are more involved in inducing cell migration and invasion.

CONCLUSIONS

This study shows that several compounds in *D. rigidula* sp. have excellent content and are predicted to have potential as breast cancer inhibitors through AKT1 protein. The results also show that compounds such as Campesterol and Stigmasterol have good binding affinity, biological activity, and toxicity tests. Additionally, the interaction of these compounds with amino acid residues on AKT1 aligns with the docking target. However, further testing is needed, especially for molecular dynamics and in-vitro cell testing.

AUTHOR CONTRIBUTIONS

DOI: 10.21009/bioma.v20i2.49340

N.M., F.N.P., S.A.E., A.D.H., S., N.R.A.: research concept; N.M.: methodology; N.M.: data analysis; N.M., F.N.P., S.A.E., A.D.H.: original manuscript writing; N.M., F.N.P., S.A.E.: editing and review; S., N.R.A.: supervision.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest in the writing of this manuscript.

DISCLOSURES AND ETHICS

Ethics approval was not required as the study used publicly available databases.

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